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(54) Diagnostic test drug comprising monoclonal antibody to human copper zinc-superoxide dismutase and diagnostic test method using the same.

(57) A measurement reagent for a human Cu· Zn-superoxide dismutase (SOD) which comprises an anti-human Cu·Zn-SOD monoclonal antibody labelled with an enzyme, a method for measuring a human Cu·Zn-SOD which comprises measuring the human Cu·Zn-SOD with the measurement reagent for the human Cu·Zn-SOD, and an anti-human Cu·Zn-SOD monoclonal antibody by the enzyme-linked immunosorbent assay according to the sandwich method, a diagnostic test drug for a human cancer of the stomach which comprises the measurement reagent and a method for diagnosing and testing a human cancer of the stomach which comprises measuring the human Cu·Zn-superoxide dismutase with the measurement reagent, and an anti-human Cu·Zn-superoxide dismutase monoclonal antibody by the enzyme-linked immunosorbent assay according to the sandwich method.

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Diagnostic test drug comprising monoclonal antibody to human copper·zinc-superoxide dismutase and diagnostic test method using the same

BACKGROUND OF THE INVENTION

This invention relates to a diagnostic test drug comprising monoclonal antibody to human copper·zinc-superoxide dismutase and a diagnostic test method using the same, and more specifically to a diagnostic test drug comprising monoclonal antibody to human copper·zinc-superoxide dismutase which is capable of conducting diagnosis of various diseases with good precision and good reproducibility and a diagnostic test method using the same.

Superoxide dismutase (SOD) is an enzyme distributed very widely in biological world and catalyzes the reaction as shown below which disproportionates the superoxide anion radicals (O_2^-) which are principal molecular species of toxic oxygen:

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

SOD is classified into the three kinds of Cu·Zn-SOD (a dimer having a molecular weight of about 32,000), Mn-SOD and Fe-SOD (both are dimers having a molecular weight of about 40,000). In the respective human

tissues, Cu·Zn-SOD is confirmed in the cytoplasm portion and Mn-SOD in the matrix portion of mitochondrion.

For Fe-SOD and Mn-SOD, high homology can be seen in their amino acid sequences between biological species and evolutional analogical relationship is estimated therebetween. In contrast, Cu·Zn-SOD is inherent in eucaryote and has a different amino acid sequence from other kinds of SOD. The Cu·Zn-SOD has been clinically recognized to have anti-inflammatory action, and the Cu·Zn-SOD derived from bovine is now under progress of development as therapeutical agent for inflammatory diseases such as chronic rheumatism. However, as a pharmaceutical, the Cu·Zn-SOD derived from human is considered to be more excellent than that derived from bovine in aspect of antigenicity. Accordingly, when an anti-human Cu·Zn-SOD antibody having very high specificity is used for purification of Cu·Zn-SOD contained in human organs (e.g. placenta) or human Cu·Zn-SOD produced in microorganism by genetic manipulation, the purification step can be by far easier than the conventional method and further still higher purity can be obtained.

On the other hand, Sawaki, Sugiura et al examined about the relationship between measured values of human serum SOD and various diseases with the use of antiserum to human SOD obtained from rabbit (reported in the 55th general meeting of Biochemical Society of Japan). According to their experimental results, the Cu·Zn-SOD value in human serum becomes some 10-fold in kidney failure, particularly uremia, and is increased to several fold in hepatitis, diabete accompanied with complications, fibroid lung, etc. The above facts indicate that Cu·Zn-SOD can be a marker for diagnosis of these diseases.

The present inventors have studied intensively in order to develop a method for conducting diagnosis of the above various diseases with good precision and good reproducibility, and consequently found that the method

in which an amount of Cu·Zn-SOD in human serum can be detected with good precision by use of monoclonal antibody specific for the human Cu·Zn-SOD can be effected for diagnosis of the cancer of the stomach to accomplish the present invention.

SUMMARY OF THE INVENTION

The present invention provides a diagnostic test drug comprising a monoclonal antibody and an enzyme-labelled monoclonal antibody recognizable human Cu·Zn-SOD (copper·zinc-superoxide dismutase) with extremely high specificity and a diagnostic test method for human Cu·Zn-SOD.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1, Fig. 2, Fig. 3 and Fig. 4 show examples of calibration curves for measurement of Cu·Zn-SOD in samples by use of the diagnostic test drug according to the present invention; Fig. 5 shows Cu·Zn-SOD concentrations in sera of various cancer patient and normal human; and Fig. 6 shows a calibration curve of Comparative example.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The monoclonal antibody to be used in the present invention can be prepared according to the method as described below.

That is, in the first place, SOD (purity: 95 % or higher) derived from human erythrocytes is immunized into, for example, BALB/c mouse, etc., and then the spleen is extirpated, followed by fusion with mouse myeloma cells of SP2 or NS-1 with the use of polyethylene glycol to obtain a hybridoma in conventional manner. And, in order to obtain a large amount of monoclonal

antibodies, a predetermined amount of the hybridoma is transplanted into the abdomen of mouse previously administered with pristane (2,6,10,14-tetramethylpentadecane), and thereafter the ascites is collected 1-2 weeks later. The ascite collected is purified by use of an ion exchange chromatograpy or ammonium sulfate fractionation. Also, the monoclonal antibody of the present invention can be prepared according to the method in which the above hybridoma is cultured in a large amount in a culturing tank.

The monoclonal antibody of the present invention thus obtained has the following properties.

1. It does not react with human albumin, human α-globulin and human γ-globulin at all.
2. It reacts specifically with a human type Cu·Zn-SOD as a matter of course, and when the liver cytosol is stained with the present antibody according to the Western blotting method, only Cu·Zn-SOD in the liver cytosol is selectively stained.

The diagnostic test drug can be used widely for immunological assay of Cu·Zn-SOD, for example, assay according to double diffusion within agar gel, one-dimensional immunodiffusion, immunoelectrophoresis, latex agglomeration, erythrocyte agglomeration reaction, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), etc.

It is possible to measure a Cu·Zn-SOD in serum or urine by use of these methods, as a matter of course. Further, cells containing Cu·Zn-SOD can be detected and diagnosed according to the method such as PAP staining, etc.

For example, to explain about the diagnostic test method according to the ELISA method, a plural kinds of purified monoclonal antibodies are mixed in appropriate amounts and apportioned into a 96-well plate for immunoassay and held at 4 ^{o}C overnight. Next, after each well

in this plate is washed with a buffer, a sample containing Cu·Zn-SOD is added and incubated at 30 °C for 3 hours. Subsequently, after each well is again washed with a buffer, a monoclonal antibody labelled with enzyme is added and incubated at 30 °C for 2 hours. Then, after washing, a substrate solution is added and after incubation for a predetermined time, absorbance at a wavelength showing maximum absorbance is measured by a spectrophotometer to determine the quantity, and at the same time, an amount of human Cu·Zn-SOD in the sample can be determined by using the prepared calibration curve.

The enzyme for use to label the human Cu·Zn-superoxide dismutase of the present invention may be mentioned, for example, peroxidase, alkalinephosphatase, β-galactosidase, catalase, glucose oxidase, lactose oxidase, alcohol oxidase and monoamine oxidase.

The monoclonal antibody to be used in the present invention can be labelled in a conventional manner (with RI, enzyme, fluorescent group, metal sol, etc.) depending on the purpose in carrying out measurement.

The diagnostic test drug according to the present invention can react specifically with Cu·Zn-SOD not only in human serum or urine but also in cytoplasm, and therefore it can be known the relationship between the amount of human Cu·Zn-SOD and various deseases of human by utilizing the monoclonal antibody. Thus, in the present invention, it is found that the measurement of the amount of human Cu·Zn-SOD utilizing the present monoclonal antibody is effective for diagnosis test of cancer of the stomach.

The present invention will be described in more detail by referring to the following Reference examples, Examples and Comparative example.

Reference example 1

Preparation of anti-human Cu·Zn-SOD monoclonal antibody

(1) Immunization

An amount of 0.25 mℓ of PBS (Phosphate Buffered

Saline) having 100 μg of human Cu·Zn-SOD dissolved therein and 0.25 mℓ of Freund's complete adjuvant were mixed to prepare an emulsion, and 0.5 mℓ of the emulsion was administered intraperitoneally to BALB/c mouse (male, 7 weeks old). Three weeks later 0.5 mℓ of an emulsion prepared by mixing 0.25 mℓ of PBS having 100 μg of the same antigen dissolved therein and 0.25 mℓ of Freund's incomplete adjuvant was administered intraperitoneally. Further, 3 weeks later, as the final immunization, 0.5 mℓ of PBS having 100 μg of the same antigen dissolved therein was administered into the tail vein.

(2) Cell fusion

On the fourth day after the final immunization, the spleen of the mouse was extirpated, washed in a laboratory dish containing Hanks' solution under ice-cooling, transferred into MEM (Eagle Minimun Essential Medium), divided into 4 equal portions and loosened by a pair of tweezers. The floating lymphocytes thus obtained were washed three times with MEM (1,000 rpm, 5 minutes) and suspended in RPMI 1640 to provide spleen lymphocytes to be used for cell fusion. Next, 2×10^7 8-AG (8-aza-guanine) resistant myeloma cells (NS-1 or SP2) previously prepared and 2×10^8 spleen lymphocytes were mixed, the supernatant was removed at room temperature (1,000 rpm, 5 minutes) and the pellet was loosened by tapping the centrifugal tube. Into this was added 1 mℓ of 45 % PEG 4000 heated to 37 °C over one minute, and the mixture was left to stand at 37 °C for 6 minutes. Then, RPMI 1640 heated to 37 °C was gradually added at the rate of 5 mℓ/min. over 3 minutes, until the mixture was finally made up to 50 mℓ, and the mixture was subjected to centrifugation at room temperature (1,000 rpm, 5 minutes), followed by removal of the supernatant. This was suspended in 200 mℓ of HAT medium previously heated to 37 °C (15 % FCS-RPMI 1640 medium containing 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, 1.6×10^{-5} M thymidine), and apportioned each in 100 μℓ to the respective wells of a 96-well

microtiter well to carry out cultivation.

(3) HAT selection

On the 4th day after cell fusion, each 50 µℓ of HAT medium was added into each well, and ELISA was conducted from about the 10th day when growth of hybridoma was recognized, and 50 µℓ of HT medium (15 % FCS-RPMI 1640 medium containing 1 x 10^{-4} M hypoxanthine, 1.6 x 10^{-5} M thymidine) was added into the well where antibody production was recognized, and thereafter those hybridomas were acclimatized in 15 % FCS-RPMI 1640 medium under observation of the growth thereof.

(4) Selection of hybridoma

Over 1 to 3 weeks from initiation of cultivation, it was assayed by ELISA whether anti-human Cu·Zn-SOD antibody was contained in the culture supernatant in the well in which cell growth was recognized.

First, into the respective wells of a 96-well U-bottomed ELISA plate, each 50 µℓ of human Cu·Zn-SOD (10 µg/mℓ) was apportioned and left to stand overnight at 4 °C. Each well was washed three times with washing liquid (PBS containing 0.1 % Tween 20), and each 50 mℓ of the above culture supernatant was apportioned thereinto and left to stand at room temperature for 2 hours (for the supernatant as the negative control, a cultured product of a mixture of spleen lymphocytes and myeloma cells before fusion cultured similarly was used). Next, these wells were washed three times, each 50 µℓ of peroxidase-labelled anti-mouse (IgG, IgM, IgA) antibody solution was apportioned into each well and left to stand at room temperature for 2 hours. These wells were washed four times, each 100 µℓ of a substrate solution (prepared by dissolving 20 mg of o-phenylenediamine and 10 µℓ of 35 % H_2O_2 in 50 mℓ of 0.1 M citrate buffer of pH 5.0) into each well and left to stand at room temperature for 30 minutes under shielding from light with aluminum foil. Finally, the enzyme reaction was stopped by apportioning each 50 µℓ of 2 N sulfuric acid into each well, and

absorbance at 492 nm was measured. Anti-human Cu·Zn-SOD antibody producing hybridoma was considered to exist in the well from which supernatant exhibiting positive enzyme activity was taken. As the result of assay of the culture supernatant in the cell grown well as described above, the (antibody producing well number/ELISA well number) was found to be (85/1566) in the case of using NS-1 as the myeloma cell and (54/329) in the case of using SP2.

(5) Establishment of hybridoma strain

By use of 15 % FCS-RPMI 1640 medium, the hybridoma in the antibody producing well as described in (4) was subjected to cloning according to the limiting dilution method. For cultivation, a 96-well microtiter well was used and 10^7/mℓ thymus cells of BALB/c mouse were used as the supporting cells, and the hybridoma was cultured at 1 cell/100 μℓ/well. From about the 10th day after cultivation, the supernatant in the well which appears to contain a single colony was sampled and subjected to ELISA by use of human Cu·Zn-SOD, and the sample in which antibody was recognized was further examined for reactivity with other antigens (human albumin, human globulin, etc.). Thus, 6 strains were selected (S-1, S-2, S-4, S-6, N-4, N-6; applied to deposition at Agency of Industrial Science and Technology, Japan on February 7, 1980 but received a Notice of refusal to receive deposition on February 13, 1980) and subjected to recloning (listed in Table 1).

(6) Determination of class·sub-class of anti-human Cu·Zn-SOD monoclonal antibody

Determination of class·sub-class of the immunoglobulin produced by each hybridoma was conducted according to the method of ELISA as described in Reference example 1 (4) by use of a peroxidase-labelled antibody (affinity purified antibody to IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgM, IgA) specific for each class·sub-class of the monoclonal antibodies produced by the 6 strains, the anti-

bodies of the two strains were IgM and those of the four strains were IgG_1 (listed in Table 1). The types of the Light chain of the respective antibodies were all κ type.

(7) Production of monoclonal antibody

Production of antibody was carried out by flask culturing or intraperitoneally in mouse. According to flask culturing, the hybridoma obtained by culturing in a 15 % FCS-RPMI 1640 medium was cultured in only RPMI 1640 immediately before death (monoclonal antibody existed in an amount of 10 to 50 μg/mℓ in the supernatant obtained by centrifugation). On the other hand, production in mouse abdomen for obtaining a large amount of antibody was carried out by administering intraperitoneally 10^6 to 10^7 hybridomas floated in RPMI 1640 into BALB/c mouse (male, 6 to 10 weeks old, administered intraperitoneally with 0.5 mℓ of pristane 2 to 3 weeks before). Marked increase of mouse body weight was recognized from about one week later, and ascites was taken out suitably after 1 to 3 weeks.

Reference example 2

Specificity of antibody

Specificity of the monoclonal antibody produced by the 6 strains as described in Reference example 1 (5) was examined from reactivities with human Cu·Zn-SOD, human Mn-SOD, bovine SOD, canine SOD, human albumin, human α-globulin, human γ-globulin, etc. (listed in Table 1). The reaction between these monoclonal antibodies with antigen was carried out according to the method of ELISA as described in Reference example 1 (4).

As shown in Table 2, everyone of the monoclonal antibodies produced by the 6 strains does neither react with albumin and globulin which are main components of human serum proteins nor react human Mn-SOD. The antibodies of the 6 strains specifically react with human Cu·Zn-SOD, and one strain (N-6) of them reacted with SOD of bovine and canine.

Table 1

Class•sub-class of monoclonal antibody

Myeloma cell used for fusion	Strain name	Class•sub-class of produced antibody	Type of L chain
SP2	S-1	M	κ
	S-2	G_1	κ
	S-4	M	κ
	S-6	G_1	κ
NS-1	N-4	G_1	κ
	N-6	G_1	κ

Table 2

Specificity of monoclonal antibody

Antigen / Antibody	Human Cu·Zn-SOD	Human Mn-SOD	Bovine SOD	Canine SOD	Human albumin	Human α-globulin	Human γ-globulin
S-1	+++	-	±	±	-	-	-
S-2	+++	-	±	±	-	-	-
S-4	+++	-	±	-	-	-	-
S-6	+++	-	-	-	-	-	-
N-4	+++	-	-	-	-	-	-
N-6	+++	-	++	+	-	-	-

(Extent of reaction between antigen and antibody: +++ > ++ > + > ± > -)

Example 1

Of the monoclonal antibodies obtained in Reference example, N-4 was used and labelled with horseradish peroxidase according to the method of NAKANE et al. (NAKANE, R.K., and KAWAOI, A.J. Histochem. Cytochem., 22, 1084, 1974).

To a 96-well immunoplate, each 100 µl of the mixed solution of monoclonal antibody (mixing ratio of S-2 : S-6 : N-4 : N-6 = 1 : 1 : 1 : 1) was added and incubated at 4 °C overnight. Next, excessive binding sites were covered with 10% bovine serum. Each 100 µl of the samples containing various concentrations of SOD was added and incubated at 30 °C for 3 hours. Next, after thoroughly washed with physiological saline, each 100 µl of the above peroxidase-labelled anti-SOD antibody appropriately diluted was added and incubated at 30 °C for 2 hours.

Further, after washing with physiological saline, activity of peroxidase was detected with o-phenylenediamine by use of hydrogen peroxide as the substrate, and absorbance was measured to obtain a calibration curve of the human Cu·Zn-SOD as shown in Fig. 1. By use of the calibration curve, SOD concentration in a sample of unknown concentration could be determined.

Also, by use of the calibration curve shown in Fig. 1, the addition recovery test of SOD was conducted to obtain the results shown in Table 3.

Table 3

Addition-recovery test of SOD

Final concentration added (ng/mℓ)	Absorbance	Reading on calibration cruve	Recovery (%)
111	0.969	110	99
36	0.599	40	110

Example 2

The anti-human Cu·Zn-SOD monoclonal antibody was bound onto a solid phase carrier of polystyrene beads, and a calibration curve was prepared similarly as in Example 1.

After polystyrene beads (produced by Wako Junyaku K.K., 3.2 mmo) were soaked in 10 % Scat 20X (trade name, produced by Daiichi Kogyo Seiyaku K.K.) for several days and thereafter washed well with tap water and distilled water. The polystyrene beads were soaked in PBS (Phosphate Buffered Saline) containing the anti-human Cu·Zn-SOD monoclonal antibody (15 μg/mℓ, S-2 : S-6 : N-4 : N-6 = 1 : 1 : 1 : 1) at 4 ^{o}C overnight to immobilize the monoclonal antibody on the polystyrene beads. Subsequently, after washing twice with PBS containing 0.1 % Tween 20 (trade name, produced by Katayama Kagaku Kogyo) and soaked in PBS solution containing 10 % CS (calf serum) to effect blocking. This was again washed and then stored in PBS solution containing 1 % BSA and 0.1 % NaN_3 to be brought into the state available at any time.

The polystyrene beads having the monoclonal antibody immobilized thereon thus prepared was taken out in necessary amount, and the same operation as in Example 1 was carried out to prepare a calibration curve of the human Cu·Zn-SOD. The calibration curve obtained is shown in Fig. 2.

Example 3

According to the same procedure as in Example 2 except for using S-2, S-6 (S-2 : S-6 = 1 : 1) as the monoclonal antibodies, calibration curve of the human Cu·Zn-SOD was prepared. The calibration curve obtained is shown in Fig. 3.

Comparative example 1

A calibration curve was attempted to be prepared according to the same procedure as in Example 2 except for using N-4 as the monoclonal antibody. However, in this case, no calibration curve could be drawn as shown

in Fig. 4.

Thus, by use of the calibration curve as obtained in Example 1, 2 and 3, the concentration of a human type Cu·Zn-SOD in a sample of unknown concentration can be determined.

Example 4

According to the same procedure as in Example 1 except for using N-4 as the monoclonal antibody and N-6 as the monoclonal antibody labelled with peroxidase by the method of NAKANE et al, calibration curve of the human Cu·Zn-SOD was prepared. The calibration curve obtained is shown in Fig. 4.

Example 5

By using sera of various cancer patients and sera of nomal men, investigation was carried out in the relations between concentrations of Cu·Zn-SOD in these sera and various deseases.

These sera employed were those in which no cythemosis was obserbed and sufficient care has been paid during preservation.

Numbers of sera of cancer patients herein used were 4 of colorectal carcinoma, 2 of cancer of the esophagus, 3 of lung cancer, 14 of cancer of the stomach and 10 of cancer of the pancreas, and numbers of sera of the normal human were 110.

The concentrations of Cu·Zn-SOD in these sera were measured by the method as described in Example 4 and a calibration curve was also prepred by the method in the same. The results are shown in Fig. 5.

As seen from Fig. 5, in the sera of the patients of cancer of the stomach, it is clearly found that quite a number of patients showing high value of the Cu·Zn-SOD concentration has been admitted as compared with the sera of the normal human. Since such high values in the Cu·Zn-SOD concentration have not been admitted in the sera of the other patients, it can be estimated that if the concentration of the Cu·Zn-SOD is high by the method

as mentioned above, he would be a cancer of the stomack. Accordingly, by the measurement of the Cu·Zn-SOD in the human serum using the monoclonal antibody specific to the human Cu·Zn-SOD, diagnostic test of the cancer of the stomack can be carried out with ease.

According to the above, it could be found that by the measurement method of the Cu·Zn-SOD using appropriate two kinds (only one of which is used by labelling with peroxidase) of the monoclonal antibodies among those specific to the human Cu·Zn-SOD of the present invention, diagnosis of the cancer of the stomack can easily be carried out. Accordingly, the fact that such a diagnosis using the monoclonal antibody can easily be carried out before carrying out the conventional medical examination by using an autoscope, an X-ray, etc., will lead to discovery of much more patients of the cancer of the stomack since increase in a number of patients to be consulted would be expected.

Claims:

1. A measurement reagent for a human Cu·Zn-superoxide dismutase comprising an anti-human Cu·Zn-superoxide dismutase monoclonal antibody labelled with an enzyme.

2. A measurement reagent for a human Cu·Zn-superoxide dismutase according to Claim 1, wherein said enzyme for use to label said human Cu·Zn-superoxide dismutase is at least one selected from the group consisting of peroxidase, alkalinephosphatase, β-galactosidase, catalase, glucose oxidase, lactose oxidase, alcohol oxidase and monoamine oxidase.

3. A method for measuring a human Cu·Zn-superoxide dismutase comprising measuring the human Cu·Zn-superoxide dismutase with a measurement reagent for the human Cu·Zn-superoxide dismutase containing an anti-human Cu·Zn-superoxide dismutase monoclonal antibody labelled with an enzyme, and an anti-human Cu·Zn-superoxide dismutase polyclonal antibody by the enzyme-linked immunosorbent assay according to the sandwitch method.

4. A method for measuring a human Cu·Zn-superoxide dismutase comprising measuring the human Cu·Zn-superoxide dismutase with a measurement reagent for the human Cu·Zn-superoxide dismutase containing an anti-human Cu·Zn-superoxide dismutase monoclonal antibody labelled with an enzyme, and another anti-human Cu·Zn-superoxide dismutase monoclonal antibody different in antigen recognizing portion from that in said measurement reagent by the enzyme-linked immunosorbent assay according to the sandwitch method.

5. A diagnostic test drug for a human cancer of the stomack comprising an anti-human Cu·Zn-superoxide dismutase monoclonal antibody labelled with an enzyme.

6. A diagnostic test drug for a human cancer of the stomack according to Claim 5, wherein said enzyme for use to label said human Cu·Zn-superoxide dismutase is at least one selected from the group consisting of peroxi-

dase, alkalinephosphatase, β-galactosidase, catalase, glucose oxidase, lactose oxidase, alcohol oxidase and monoamine oxidase.

7. A method for diagnosing and testing a human cancer of the stomack comprising measuring the human Cu·Zn-superoxide dismutase with a measurement reagent for the human Cu·Zn-superoxide dismutase containing an anti-human Cu·Zn-superoxide dismutase monoclonal antibody labelled with an enzyme, and an anti-human Cu·Zn-superoxide dismutase polyclonal antibody by the enzyme-linked immunosorbent assay according to the sandwitch method.

8. A method for diagnosing and testing a human cancer of the stomack comprising measuring the human Cu·Zn-superoxide dismutase with a measurement reagent for the human Cu·Zn-superoxide dismutase containing an anti-human Cu·Zn-superoxide dismutase monoclonal antibody labelled with an enzyme, and another anti-human Cu·Zn-superoxide dismutase monoclonal antibody different in antigen recognizing portion from that in said measurement reagent by the enzyme-linked immunosorbent assay according to the sandwitch method.

FIG. 1

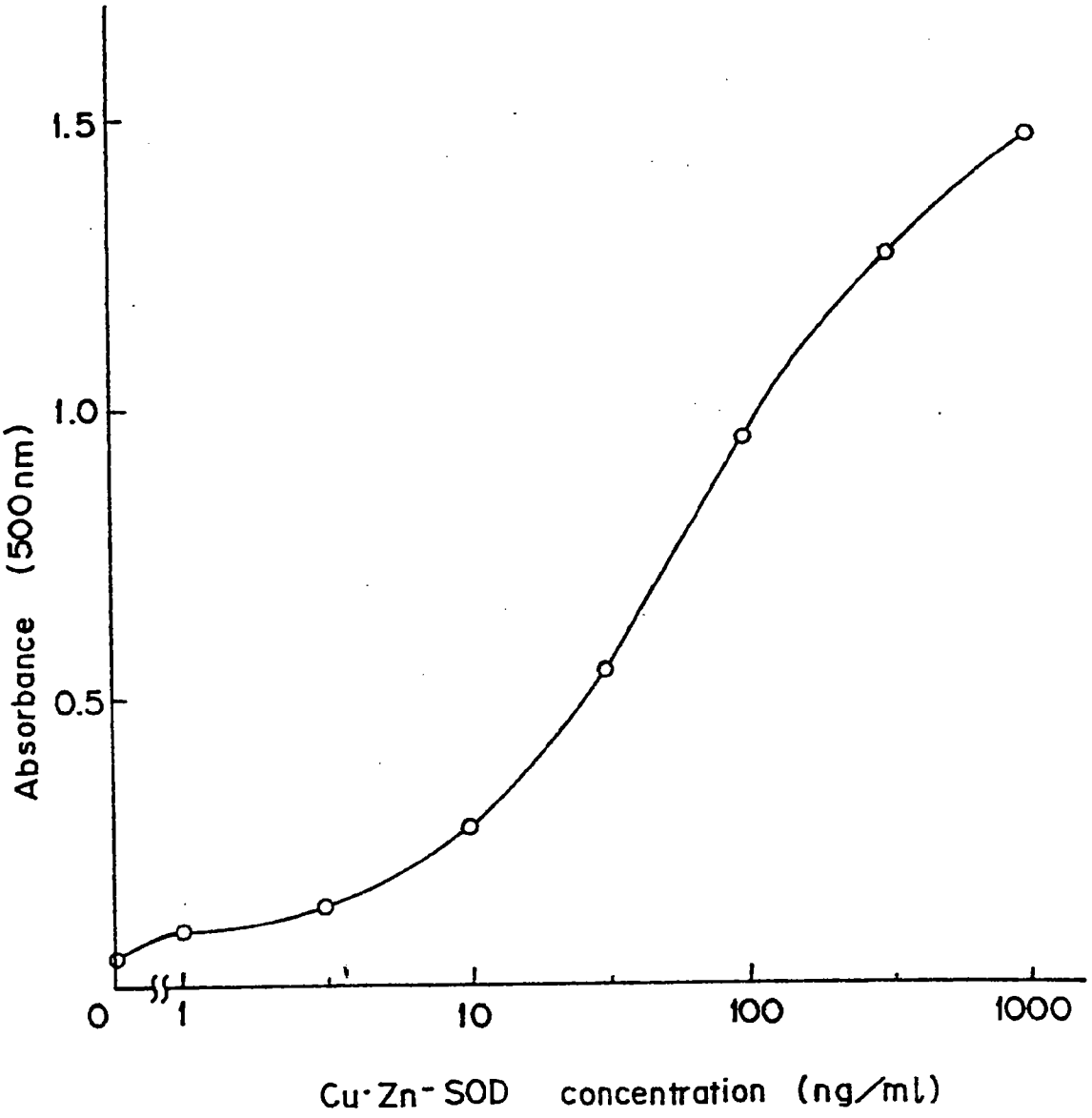
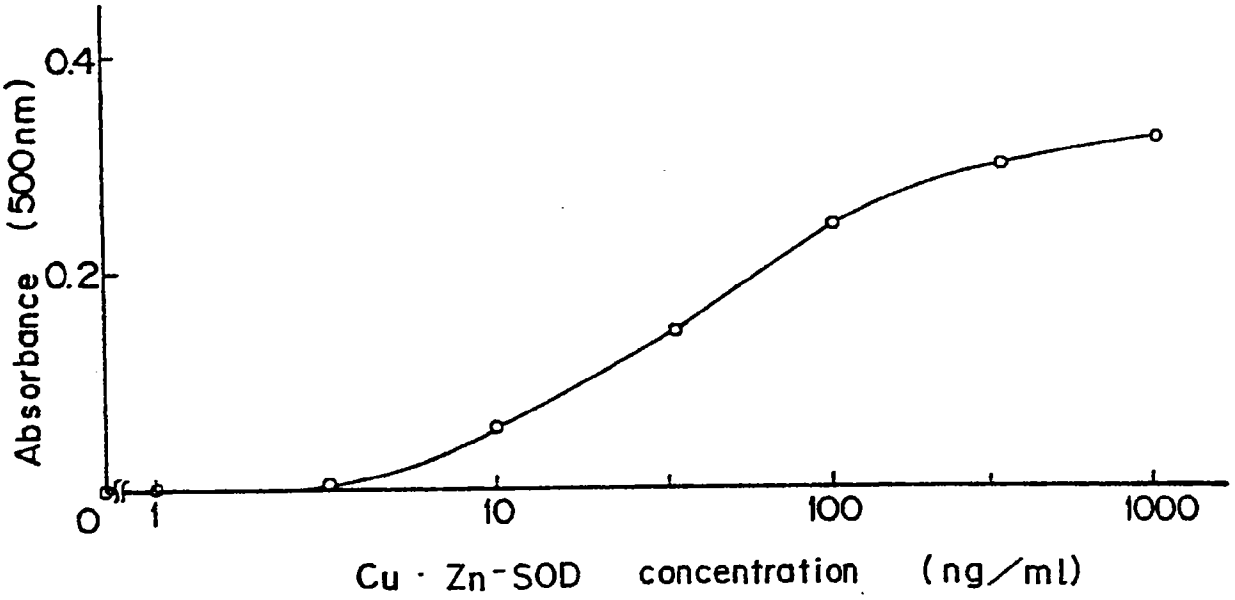

1.5
1.0
0.5
0
Absorbance (500nm)
1
10
100
1000
Cu·Zn-SOD concentration (ng/ml)

FIG. 2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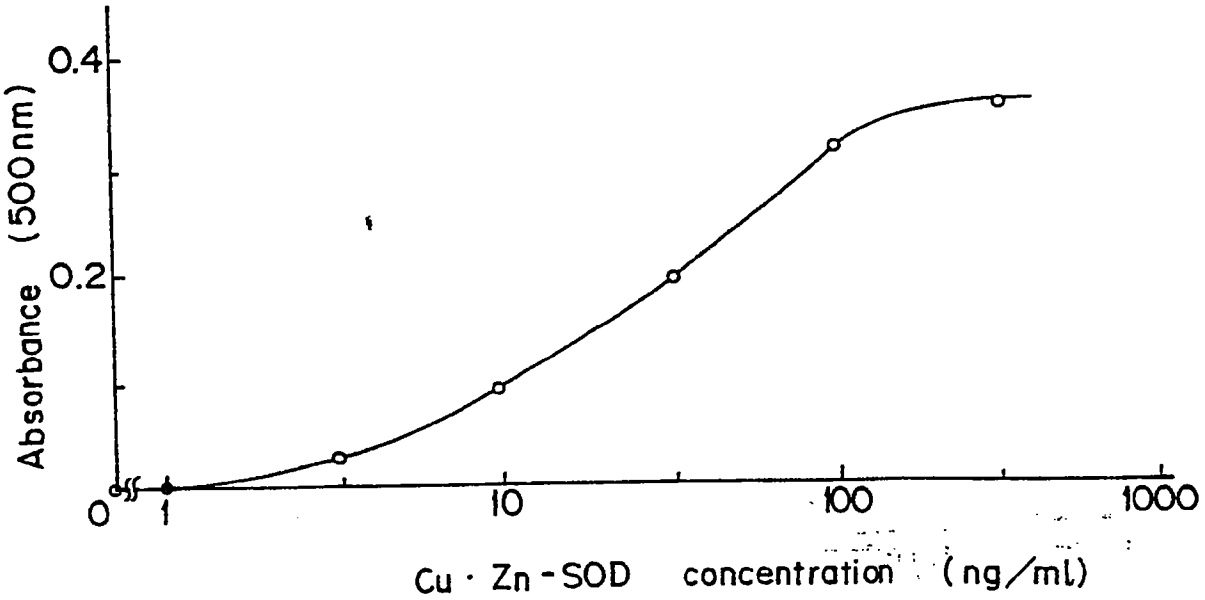
0.2
0
Absorbance (500nm)
1
10
100
1000
Cu · Zn-SOD concentration (ng/ml)

FIG. 3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0.2
0
Absorbance (500nm)
1
10
100
1000
Cu · Zn-SOD concentration (ng/ml)

FIG. 4

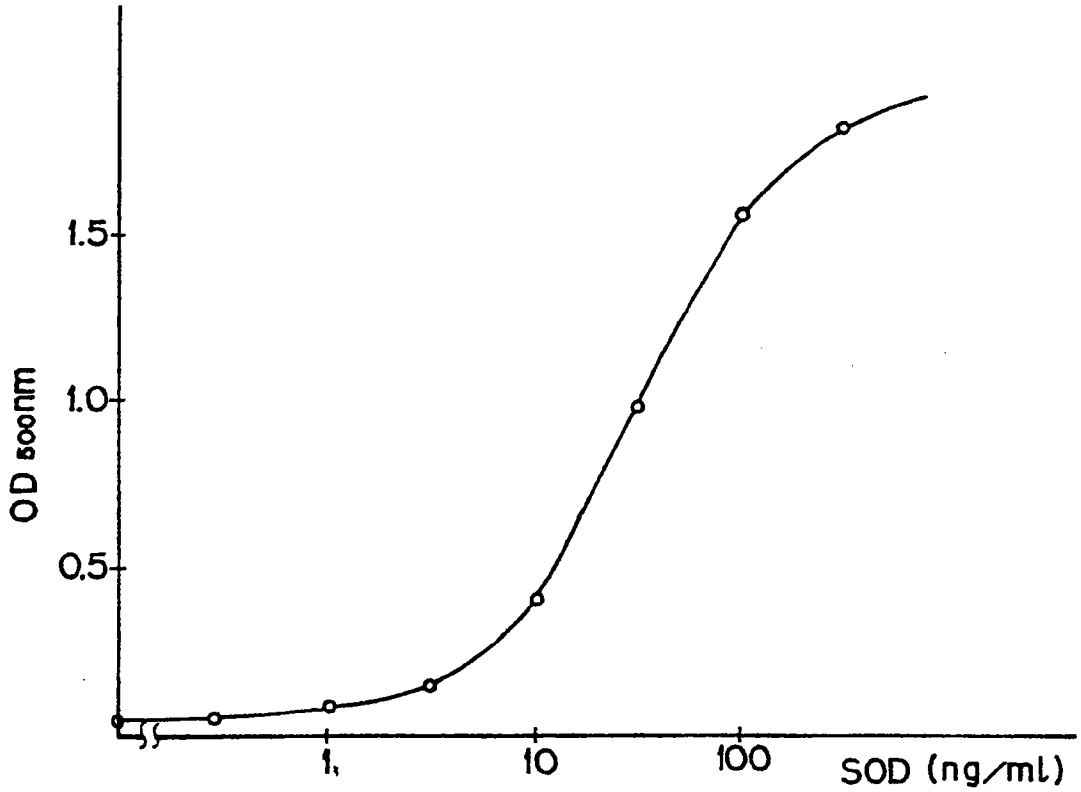

FIG. 5

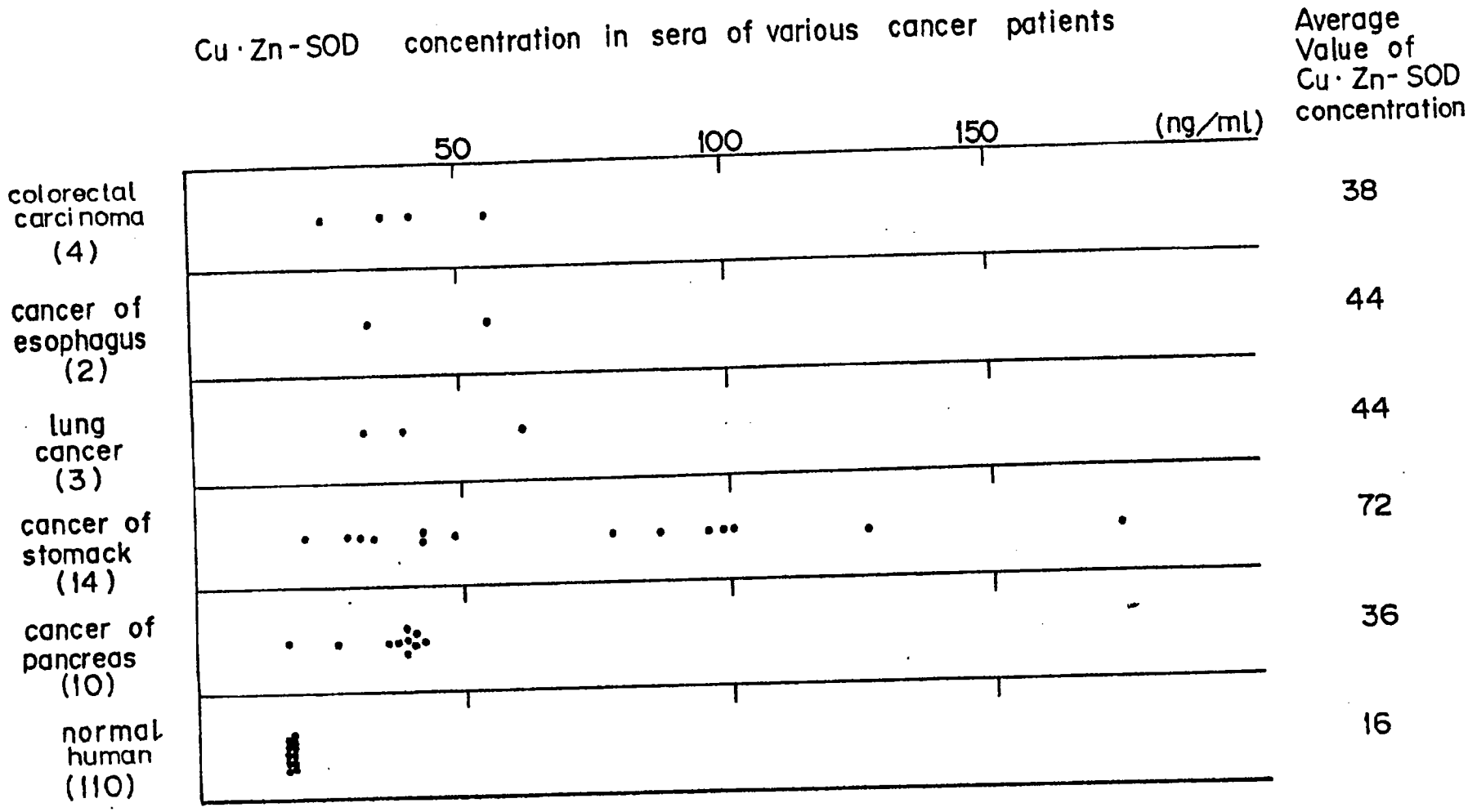

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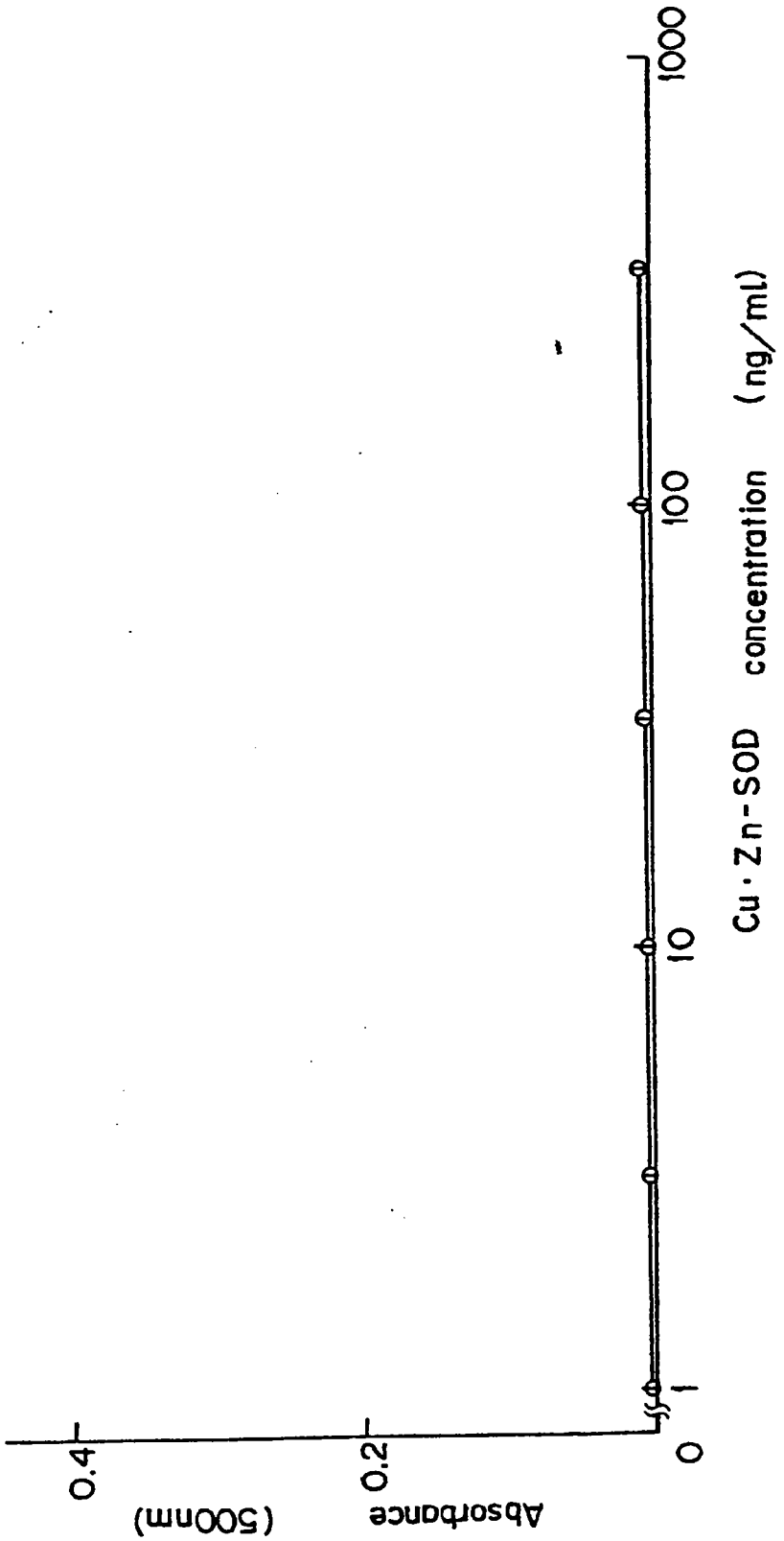
FIG. 6


Absorbance (500nm)
0.4
0.2
0
1
10
100
1000
Cu·Zn-SOD concentration (ng/ml)

European Patent Office

0217542

Application number

EP 86 30 6670

EUROPEAN SEARCH REPORT

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	PATENTS ABSTRACTS OF JAPAN, vol. 9, no. 254 (C-308)[1977], 11th October 1985; & JP-A-60 109 528 (FUJISAWA YAKUHIN KOGYO K.K.) 15-06-1985 * Abstract * & Chemical Abstracts, vol. 103, no. 21, 25th November 1985, page 560, abstract no. 176.901d	1,3-5, 7,8	C 12 Q 1/26 G 01 N 33/573 G 01 N 33/574
Y	PATENTS ABSTRACTS OF JAPAN, vol. 7, no. 78 (P-188)[1223], 31st March 1983; & JP-A-58 7560 (MAMORU SUGIURA) 17-01-1983	1,3-5, 7,8	
X		1,2,5, 6	
Y	GB-A-2 089 979 (TOYO JOZO CO. LTD.) * Abstract; page 2, lines 7-28; pages 2-4; example 1 *	1,3-5, 7,8	TECHNICAL FIELDS SEARCHED (Int. Cl.4) G 01 N C 12 Q
X		1,2,6	

-/-

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	21-10-1986	MEYLAERTS H.

CATEGORY OF CITED DOCUMENTS

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EUROPEAN SEARCH REPORT

Application number

EP 86 30 6670

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	CHEMICAL ABSTRACTS, vol. 101, no. 5, 30th July 1984, page 206, abstract no. 34900n, Columbus, Ohio, US; S. IIZUKA et al.: "Enzyme-linked immunosorbent assay for human manganese-containing superoxide dismutase and its content in lung cancer", & JNCI, J. NATL. CANCER INST. 1984, 72(5), 1043-9 * Abstract *	1-8	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	21-11-1986	MEYLAERTS H.

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